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Vermiculite, a Source of Metals for 'Arabidopsis thaliana'

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16. ABSTRACT

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Vermiculite, a Source of Metals for *Arabidopsis thaliana*[1]

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JOURNAL ARTICLE

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Abstract. **Plants of *Arabidopsis thaliana* (L.) Heynh. were grown in hydroponic systems using vermiculite as a growth medium at nutrition levels ranging from adequate to deficient. Plants grown on the low-total nutrient or low-iron nutrient contained more of iron, magnesium, and aluminum, frequently twice as much as plants grown at normal nutrient levels. Plants grown in iron-deficient nutrient were initially chlorotic; after about 5 weeks the chlorosis disappeared and these plants contained higher tissue iron levels than those receiving iron in the solutions. Concentrations of aluminum in the foliage ranged between 2 and 5 mg/g dry weight. Chemical analysis of the nutrient solution indicated that there was not sufficient aluminum, as a contaminant, in the nutrient solution to account for the concentrations that occurred in the plant tissue. Apparently, plants experiencing nutrient stress may modify the root environment so that vermiculite is solubilized and iron, magnesium, and aluminum are taken up by the plant. Levels of aluminum that accumulate in *Arabidopsis* foliage suggest that it should be classified as an aluminum-accumulating plant.**

Arabidopsis, an annual herbaceous member of the Cruciferae, has gained considerable attention as a research tool and is favored by more botanists for instructional purposes than other angiosperms (12). Preliminary experiments to develop a bioassay for toxic substances using *Arabidopsis* in a vermiculite-hydroponic cultural system indicated that the plants contained substantially higher concentrations of calcium (Ca), magnesium (Mg), and iron (Fe) than are typically found in plants (8). Elevated concentrations of aluminum (Al) were also detected. Vermiculite is a common plant growth subtrate used extensively in horticulture because it has high water-holding capacity, good porosity, large cation exchange capacity, and base exchange, providing buffering in the root zone (1, 7). It contains substantial amounts of Si, Al, Fe, Mg, and Ca (Table 1).

Studies were conducted to determine the source of the high metal concentrations in plant tissue grown on vermiculite by varying the concentrations of nutrients in the hydroponic system.

Methods and Materials

Seeds of Columbia wild type arabidopsis were obtained from Dr. G. P. Rédei, University of Missouri, Columbia. To obtain uniform distribution, seeds were suspended in an agar solution (0.15%) and dispersed onto the vermiculite surface with a separatory funnel (12). Plants were cultured in a double container vermiculite-hydroponic system in which the bottom reservoir contained the nutrient solution, and the upper one (surface area 896 cm^2, vermiculite depth 13 cm) contained the growth medium (vermiculite #2 grind topped with about 1 cm of #3 grind) and all the roots (Fig. 1). Nutrient solution was transferred into the upper reservoir via capillary action in polyester wicking. In concurrent studies, gravimetric measurements indicated that the growth medium was maintained at field capacity throughout the study. Seedlings were cultured until harvest in a greenhouse under a 16-hr light, 8-hr dark photoperiod. Sunlight was supplemented with high-intensity discharge sodium vapor lamps (400W) yielding a mean maximum midday photosynthetic photon flux density of 350 ± 50 $\mu E\ m^{-2} s^{-1}$, and mean maximum day/night temperatures averaged 29° ± 3° and 20° ± 1°C, respectively. Photosynthetic photon flux density at canopy height (90 cm below the lights) was measured with a LI-COR Quantum Sensor, Model LI-190SR (LI-COR Inc., Lincoln, Neb.).

Plants were grown at about 1 plant/4 cm^2 and nutrient solution was added to the lower container as needed. Plants were harvested about 60 days after seeding by first collecting mature seeds and then harvesting the remaining aboveground (foliage) plant material. The mature seeds were air-dried and plant material was oven-dried at 70°C to constant weight. Following dry weight determination, the plant material was ground in a Wiley Mill to pass a 40-mesh screen and analyzed for mineral content. Seeds for tissue analysis were ground with a porcelain mortar and pestle. Aliquots of plant material were digested with $HClO_4$ (10). For mineral analysis, vermiculite was solubilized by a $LiBO_2$ fusion (11). Silicates were determined by atomic absorption analysis (11), and metals in the study were determined with an inductively coupled plasma atomic emission spectrometer (10). To estimate interstitial water pH, a vermiculite core (5 cm diameter) was collected and centrifuged, and the pH was measured on the supernatant above the pellet.

Metal accumulation was studied in two separate experiments. In the first, plants were grown at 3 levels of total nutrition, 1/2, 1, and 2 times the standard (1 ×) North Carolina State University Phytotron nutrient solution (6). In the second study, the plants received a normal level (1 ×) of all nutrients except Fe, which was varied from 0 to 10 mg/liter Fe as the diethylene triamine

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Table 1. Partial elemental composition of vermiculite.[z]

Element	Elemental Composition (%)	
	1	2
Si	18.1	18.4 ± 0.03
Mg	13.7	13.0 ± 0.03
Al	7.9	6.9 ± 0.10
Fe	6.5	8.44 ± 0.02
Ca	0.88	1.04 ± 0.03
Mn	0.08	0.09 ± 0.002

[z]Data in column 1 from reference 1. Data in column 2 from the chemical analyses of vermiculite used in the study. The analyses were performed to confirm published values (1). Each mean is based on 3 observations.

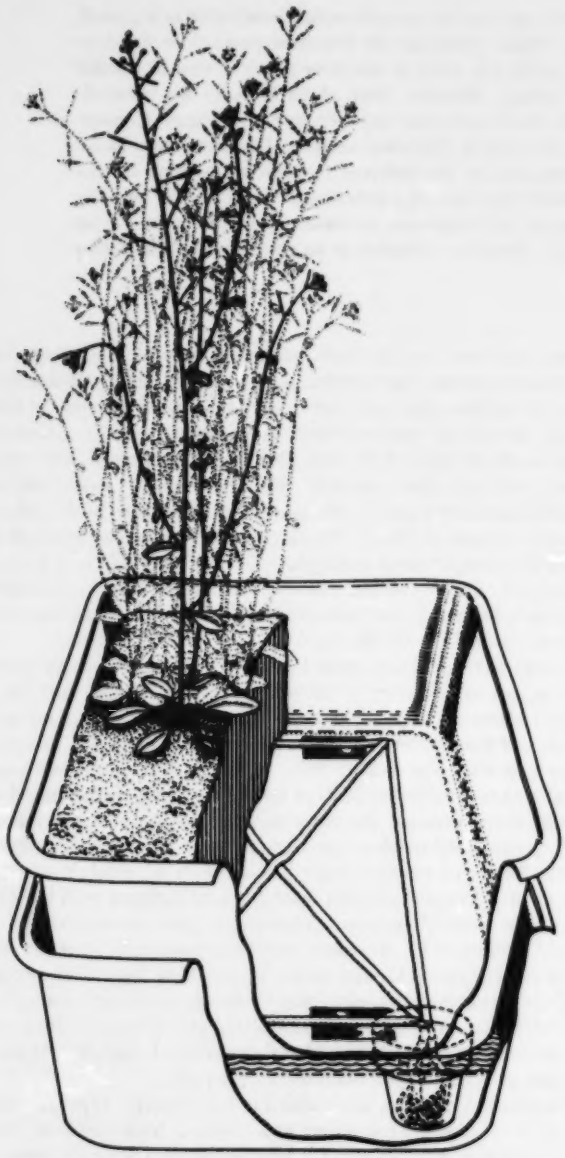

Fig. 1. The vermiculite-hydroponic double-container plot cultural system with an example of *Arabidopsis thaliana*.

pentaacetate (Sequestrene 330) chelate. In both studies, fresh nutrient solution was added to the lower reservoirs as needed to maintain water and nutrient levels.

To stabilize the variance, data for plant growth and metal content were transformed to their respective logarithms prior to analysis (14). To test for a significant treatment effect, i.e., did nutrient strength or Fe concentration influence plant growth or the concentration of metals within the tissue, the log of each respective response was regressed against the log of either the nutrient concentration or the Fe concentration. In the Fe study the 0 mg/liter concentration was set at 0.05 prior to log transformation. A significant slope for a regression equation was interpreted as a significant treatment effect on the response. Linear regression was used for the nutrient concentration study, and a quadratic regression approach was used in the Fe concentration study. Data are presented as geometric means and standard geometric errors (SGE). The SGE is calculated as $_{e}s/\sqrt{n}$, where s is the standard deviation of the response in the natural logarithm scale.

Results and Discussion

Plant dry weight at the 1× and 2× nutrient levels were similar, but decreased significantly (50%) when the nutrient concentration was decreased to 0.5× (Table 2). Calcium concentration in the tissue also decreased as the solution nutrient concentration was decreased. In contrast, when the nutrient levels of all nutrients were decreased from 1 to 0.5×, tissue concentrations of Fe, Mg, and Al increased about 58, 34, and 91%, respectively.

When the Fe concentration was reduced from 5 to 0 mg/liter in the nutrient solution, plant growth decreased by about 50% (Table 3). Associated with the decreased plant growth were significant increases in the tissue content of Ca (20%), Fe (84%), Mg (54%), and Al (112%). These increases in Fe, Mg, and Al were greater than could be explained by reduced growth and resultant concentration of the metals in the tissue. Plants receiving less than 5 mg/liter Fe in the nutrient solution appeared smaller and flowered later. Also, the number of chlorotic plants was inversely related to the Fe concentration in the nutrient solution. However, within 35 days from seeding, all the plants had flowered and those plants in the low Fe concentration solutions were no longer chlorotic.

The vermiculite used in the studies was leached with nutrient solution and analyzed for leachable Al. The Al concentration in the leachate was equivalent to the concentration in the applied nutrient solution. The Al concentration of the nutrient solutions

Table 2. Effect of various nutrient solution concentrations on arabidopsis growth and metal concentration.[z]

Nutrient solution level	Plant dry wt (g)	Tissue concn (mg/g dry wt)			
		Ca	Fe	Mg	Al
0.5×	18.40	13.48	3.27	12.36	4.88
1.0×	36.98	18.88	2.07	9.23	2.56
2.0×	39.96	20.08	2.20	9.04	2.02
SGE	1.02	1.03	1.24	1.13	1.22

[z]Each geometric mean is based on pooled data from 225 ± 20 plants in each of 2 hydroponic systems for each nutrient level. The SGE (standard geometric error) is calculated as $_{e}s/\sqrt{n}$, where s is the standard deviation of the response in the natural logarithm scale. Data analysis indicated significant (5%) treatment responses for all variables. The 1.0× nutrient level was the normal strength phytotron nutrient solution (6).

Table 3. Effect of iron nutrition on arabidopsis growth and metal concentration.[z]

Fe in nutrient solution, (mg/liter)	Plant dry wt (g)	Tissue concn (mg/g dry wt)			
		Ca	Fe	Mg	Al
0.0	27.38	26.25	3.46	11.38	3.60
1.25	31.21	20.63	2.14	8.81	2.64
2.5	36.87	20.82	1.44	6.70	1.63
5.0	40.43	21.79	1.88	7.41	1.70
10.0	36.63	21.99	1.84	7.29	1.86
SGE	1.02	1.03	1.05	1.03	1.02

[z]Each geometric mean is based on pooled data from 240 ± 22 plants in each of 2 hydroponic systems for each Fe level. The SGE (standard geometric error) is calculated as $_e s/\sqrt{n}$, where s is the standard deviation of the response in the natural logarithm scale. Regression analysis indicated significant (5%) changes in all responses with Fe nutrition. Plants were grown in North Carolina State University Phytotron nutrient solution (6) which normally contained 5 mg l^{-1} Fe.

Table 4. Effect of iron nutrition on the metal concentration of arabidopsis seed.[z]

Fe in nutrient solution, (mg/liter)	Seed content			
	Ca (mg/g)	Fe (μg/g)	Mg (μg/g)	Al (μg/g)
0.0	4.39	150.6	2.69	267.3
1.25	4.88	137.4	2.77	109.2
2.50	4.88	138.1	2.74	132.3
5.0	4.80	137.2	2.84	98.4
10.0	4.89	131.7	2.76	112.1
SGE	1.034	1.080	1.018	1.066

[z]Each geometric mean is based on pooled data from seeds collected from 240 ± 22 plants in each of 2 hydroponic systems per Fe level. the SGE (standard geometric error) is calculated as $_e s/\sqrt{n}$, where s is the standard deviation of the response in the natural logarithm scale. Data analysis indicated significant (5%) treatment responses for all variables. The 1.0× nutrient level was the normal strength phytotron nutrient solution (6). Seed were collected from the plants shown in Table 3. Regression analysis indicates a signficant (5%) change in only the Al content with iron nutrition.

used in all studies averaged 0.17 ± 0.04 mg/liter. About 60 liters of nutrient solution were added to each hydroponic container during the course of the studies, and if all the solution's Al was readily available and accumulated in the foliage, approximately 10.2 mg Al would occur in the foliage from each hydroponic system. However, the Al concentration of the plant foliage in the nutrient study (Table 2) averaged about 90 to 103 mg per hydroponic container for the 0.5 and the 1.0× treatments, respectively. Similarly, in the Fe study, the Al concentration in the plant foliage averaged 99 and 69 mg per hydroponic system for the 0 and 5 mg/liter treatments, respectively. The Al concentration of the plant foliage alone in both studies was much greater than could be accounted for by the Al contamination of the nutrient solution.

To determine if the high levels of Fe, Mg, and Al observed in the tissue (Tables 2 and 3) resulted from possible vermiculite contamination of the plant samples prior to analysis, seeds from the Fe study were collected, cleaned, and analyzed for metals (Table 4). The concentrations of Ca, Fe, and Mg in the seeds were not significantly different at any level of Fe nutrition. In contrast, the Al content of the seeds from the 0 mg/liter Fe treatment was significantly higher (172%) than from the 5 mg/liter treatment. The dissimilar trends of major vermiculite constituents, Fe, Mg, and Al, in the seed argues against vermiculite contamination of the plant samples and supports the concept of the plant uptake of the metals from the vermiculite growth medium.

Vermiculite mineral is particularly susceptible to acid decomposition (3) and begins to decompose at about pH 5.5 with significant decomposition below pH 5, releasing and solubilizing Al (9, 13). Measurements of the interstitial water pH at the end of the experiments indicated that the pH ranged between about 6.0 and 6.5. At these pH ranges, one would expect Al to be essentially insoluble (9, 13), and hence unavailable for plant uptake; however, significant Al concentrations occurred within the plant tissue.

When plants are subjected to Fe stress, the Fe-efficient genotypes respond by releasing hydrogen ions and reducing agents from the roots, reducing ferric to ferrous ions at their roots, and increasing organic acids within the roots (4). It appears that arabidopsis is an Fe-efficient plant. When grown in the absence of Fe in the nutrient solution, the plants were initially chlorotic, but after several weeks the chlorosis disappeared and at harvest the plant tops contained a higher concentration of iron than did those receiving iron in the nutrient solutions. If arabidopsis were behaving as an Fe-efficient plant, its roots would release hydrogen ions and reductant into the soil solution, causing localized pH decreases near the roots. This decrease may affect a small area and not be detected in the bulk interstitial water pH measurements. A localized pH decrease could solubilize the vermiculite growth medium, increasing Fe availability to the plant. This reaction would explain loss of chlorosis with time and the high Fe content measured in both studies at low Fe levels (Tables 2 and 3). Associated with the solubilization of Fe from the vermiculite would be an increased availability of Mg and Al. Previous studies using vermiculite in hydroponic systems indicated that Mg did not need to be included in the nutrient solution because it was released from the vermiculite (2). The high levels of Fe, Mg, and Al in the plant tissue were apparently a consequence of a plant modification of the root zone in response to nutrient stress, with the concurrent solubilization of small amounts of vermiculite and subsequent release of its metals for plant uptake. Similarly, studies with lunar soil samples showed that they did not contain water soluble forms of Fe or Al; however, cruciferous species contained high tissue levels of these metals, indicating that plants could dissolve and absorb metals from insoluble forms (15).

In both studies, the Al concentrations in arabidopsis foliage from normal nutrient solutions were about 2 mg/g and 3.5 to 5 mg/g in stressed plants (Tables 2 and 3). Plants that contain more than 1 mg/g Al in the foliage are known as aluminum accumulators (5). It would appear that arabidopsis should be classified as an aluminum-accumulating species, even though it does not exhibit many of the morphological features associated with aluminum accumulating species (5).

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